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(57) Abstract

An immunogenic composition comprising an immunogen solubilised, or otherwise distributed, in a hydrophobic solvent in the absence of a hydrophilic phase. Preferably, the immunogenic composition is provided as an oral vaccine.

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IMMUNOGENIC COMPOSITIONS

The present invention relates to immunogenic compositions comprising an immunogen dissolved in a hydrophobic solvent in which it would not normally be soluble, to processes for preparing these compositions and to their use in the treatment or prophylaxis of disease, or use in the preparation of agents for such use. In particular, the invention relates to immunogenic compositions useful as oral vaccines.

For many applications, e.g in the pharmaceutical sciences, in food technology or the cosmetics industry, work with proteins and similar macromolecules presents problems because their hydrophilicity and high degree of polarity limit the extent to which they can interact with or incorporate into lipid phases. Many natural systems employ lipidic barriers (eg skin, cell membranes) to prevent access of hydrophilic molecules to internal compartments; the ability to disperse proteins in lipidic vehicles would open up a new route to introduction of these macromolecules into biological systems, whereby the lipid medium containing the protein can integrate with the hydrophobic constituents of barriers, instead of being excluded by them.

Another area where solubilisation of hydrophilic macromolecules in hydrophobic solvents would be useful is the area of compositions designed to elicit an immune response, e.g. vaccines, and in particular oral vaccines. Vaccines rely for their effectiveness in producing an immune response in a host to a specific antigen or group of antigens. Very often the antigenic component or components of a vaccine comprises one or more proteins,

for example viral coat proteins. Administration of the vaccine to a subject induces antibody production in the subject leading to protection against infection by the particular agent from which the antigen or antigens were derived.

However, vaccines for oral administration which comprise proteins will have problems associated with them for the reasons discussed above. Thus, there exists a continuing need for oral vaccines which are capable of inducing the appropriate immune response when administered to a subject, but which are not subject to these problems.

It has now been found that such immune responses can be induced by the use of preparations where an immunogen is dissolved in a hydrophobic solvent such as an oil. In particular proteins or peptides solubilised in oils can be used to generate immune responses and therefore are useful in the preparation of vaccines, particularly oral vaccines.

Dispersion of hydrophilic substances in oil phase rather than aqueous media confers other benefits in terms of increasing their stability with respect to temperature-mediated denaturation, hydrolysis, light sensitivity etc. Oils can be chosen which remain fluid over a wider temperature range than aqueous solutions, or that have a higher viscosity, resulting in greater protection against physical damage. In mixed-phase systems, sequestration of proteins in oil can limit mutually harmful interactions - eg oxidation or hydrolysis - with water-soluble compounds.

There are examples of formulations containing both

macromolecules and oil and one such example is disclosed in EP-A-0366277. The formulation disclosed in this document is an emulsion having both a hydrophobic and a hydrophilic phase, wherein the hydrophobic phase contains chylomicra or chylomicron-forming lipids. However, the macromolecule is dissolved in the hydrophilic phase not in the hydrophobic phase.

EP-A-0521994 also relates to a composition suitable for the oral delivery of macromolecules which comprises a biologically active material in association with lecithin or a compound capable of acting as a precursor for lecithin *in vivo*. All of the compositions exemplified are formulations which comprise a hydrophilic and a lipophilic phase. Once again, in this prior art document, the macromolecule is dissolved in the hydrophilic phase rather than in the lipophilic phase.

Although the formulations mentioned above do contain both macromolecules and oils, it is significant that in all cases the macromolecule is dissolved in the hydrophilic rather than in the lipophilic phase. Attempts to form true solutions of macromolecules in oils have met with limited success, and no examples of such solutions for use in vaccines are known.

US-A-5340588 discloses vaccine preparations based on lipospheres which are solid at room temperature. They have a layer of phospholipid embedded on the surface of a liposphere core and the antigen, or immunogen is either located in the core, can be attached to or within the phospholipid or both.

UK patent application No. 9323588.5 discloses a process

by which a hydrophilic species can be solubilised in a hydrophobic solvent in which it would not normally be soluble. The process relies on the surprising discovery that if a hydrophilic species is mixed with an amphiphile under certain conditions, the resultant composition will be readily soluble in lipophilic solvents such as oils.

The present invention relates to the surprising discovery that an immunogen solubilised in a lipophilic solvent such as an oil, can induce an immunogenic response in a subject to which it is administered. In particular oral administration is facilitated.

This lipidic vehicle could also act as a more effective particulate delivery system, being readily taken up by phagocytosis.

Therefore, in a first aspect the present invention provides an immunogenic composition comprising an immunogen solubilised, or otherwise distributed, in a hydrophobic solvent in the absence of a hydrophilic phase. In a preferred embodiment, the immunogen is an antigen and the composition is a vaccine, e.g. an oral vaccine.

As used herein, the term "immunogen" relates to a species capable of eliciting an immune outcome. This outcome can be a typical immune response, e.g. production of antibodies, or the triggering of differentiation or expansion of specific populations of T cells, and can be systemic or local, e.g. restricted to a mucosal response. Alternatively, the immune outcome can be, for instance, immune tolerance, in which the naive immune system is rendered unresponsive to challenge by a specific antigen.

Another alternative outcome may be desensitisation, in which a pre-existing tendency to an autoimmune or allergic response (IgE) against a specific antigen is reduced

Examples of suitable immunogens include Diphtheria toxoid, tetanus toxoid, botulin toxoid, snake venom antigens, Hepatitis B antigens, whooping cough subunit, influenza a and/or b (whole killed virus or protein subunits), cholera antigens, *H. pylori* antigens, whole bacteria, or extracts thereof, e.g. *P. aeruginosa*, *chlamydia* species, *neisseria* species, *yersinia* species, *salmonella* species, fungi or fungal antigens, e.g. from Candida, rabies virus, polio virus, rotavirus, measles virus, rubella, respiratory synctitial virus, HIV, BCG, other mycobacterial antigens, protozoal antigens, e.g. malaria, leishmania, toxoplasma, trypanosoma, trematode antigens, e.g. schistosoma, cestode antigens, e.g. from cysticerca, echinococcus, nematode antigens, e.g. toxocara, hookworm and filaria, spirochete antigens, e.g. *borrelia* species, surface membrane epitopes specific for cancer cells, and cell receptor targeting anti-inflammatory immunomodulators (for treatment of inflammatory diseases such as Crohn's Disease and rheumatoid arthritis), e.g anti TNF-R and anti IL-1R.

Non-protein antigens may be used, such as, for example, polysaccharides or polymer conjugates of steroids.

One advantage of the present invention is that different antigens (e.g. proteins and polysaccharides) can be co-presented together in the same vehicle to elicit an enhanced immune response by virtue of one component acting as a carrier for the other, without the need for

any covalent linkage.

Examples of antigens which can be used to reduce or eliminate an immune response against them include HLA antigens and polynucleotides such as RNA, and DNA, including plasmid DNA.

Examples of antigens which can be used to elicit a desensitising immune response include pollens, dust mite antigens, bee stings and food allergens.

It is also possible, where the immunogen is a peptide, polysaccharide or other antigen, to conjugate it with at least one medium- or long-chain hydrocarbon tail.

In another embodiment, the immunogen is co-solubilised with one or more cytokines in order to enhance the response. Examples of suitable cytokines include IL-4, IL-10, IL-12 and γ-interferons. Other immuno-stimulants may also be incorporated, for example, monophposphoryl lipid A, hydrobacterial extracts, muramyl dipeptide and analogues, tuftsin and cholera subunit B and heat labile toxin of *E. coli*.

In a second aspect the invention provides a process for the preparation of an immunogenic composition which comprises the step of solubilising or otherwise distributing an immunogen in a hydrophobic solvent in which it would not normally be soluble. Preferably the composition will be an oral vaccine.

The processes disclosed in UK Patent Application No. 9323588.5 are particularly suitable for solubilising immunogens in hydrophobic solvents for use in the

preparation of the compositions of the present invention.

Thus, in a preferred embodiment of this aspect of the invention the process for solubilising the hydrophilic species comprises the following steps:

(i) associating the immunogen with an amphiphile in a liquid medium;

(ii) removing the liquid medium to leave an array of amphiphile molecules with their hydrophilic head groups orientated towards the immunogen and wherein there is no chemical interaction between the amphiphile and the immunogen; and

(iii) providing a hydrophobic solvent around the immunogen/amphiphile array.

There are numerous amphiphiles which may be used in the present invention and zwitterionic amphiphiles such as phospholipids are among those which have been found to be especially suitable. Phospholipids having a phosphatidyl choline head group have been used with particular success and examples of such phospholipids include phosphatidyl choline (PC) itself, lyso-phosphatidyl choline (lyso-PC), sphingomyelin, derivatives of any of these, for example hexadecylphosphocholine or amphiphilic polymers containing phosphoryl choline and halogenated amphiphiles, e.g. fluorinated phospholipids. In the present application, the terms phosphatidyl choline (PC) and lecithin are used interchangeably. Suitable natural lecithins may be derived from any convenient source, for example egg and, in particular, soya. In most cases, it is preferable to select an amphiphile which is chemically

similar to the chosen hydrophobic solvent and this is discussed in greater detail below. Octyl glucosides, α-tocopherol, or esters thereof, or indeed mixtures of any of the above, can also be used.

The fact that the present inventors have found zwitterionic amphiphiles such as phospholipids to be particularly suitable for use in the process is a further indication of the significant differences between the present invention and the method of Okahata *et al*. Significantly, the authors of that prior art document concluded that anionic and zwitterionic lipids were completely unsuitable for use in their method and stated that they obtained zero yield of their complex using these lipids.

The hydrophobic solvent of choice will depend on the type of species to be solubilised and on the amphiphile. Suitable solvents include hydrocarbons, e.g. non-polar oils such as mineral oil, squalane and squalene, long chain fatty acids with unsaturated fatty acids such as oleic and linoleic acids being preferred, alcohols, particularly medium chain alcohols such as octanol and branched long chain alcohols such as phytol, isoprenoids, e.g. nerol, and geraniol, other alcohols such as t-butanol, terpineol, monoglycerides such as glycerol monooleate (GMO), other esters, e.g. ethyl acetate, amyl acetate and bornyl acetate, medium- or long-chain mono-, di- or tri-glycerides and mixtures thereof, halogenated analogues of any of the above including halogenated oils, e.g. long chain fluorocarbons and iodinated triglycerides, e.g. lipidiol.

Optimum results are generally obtained when the

hydrophobic solvent and the amphiphile are appropriately matched. For example, with a solvent such as oleic acid, lyso-PC is a more suitable choice of amphiphile than PC, whereas the converse is true when the hydrophobic solvent is a triglyceride.

In addition, in some cases it has been found to be advantageous to add a quantity of the amphiphile to the hydrophobic solvent before it is brought into contact with the immunogen/amphiphile array. This ensures that the amphiphile molecules are not stripped away from their positions around the hydrophilic species because of the high affinity of the amphiphile for the hydrophobic solvent.

The orientation of amphiphile molecules into an array with their hydrophilic head groups facing the moieties of an immunogen can be achieved in several ways and examples of particularly suitable methods are discussed in more detail below.

In a first method, which has a similar starting point to the method described by Kirby *et al*, *in Bio/Technology*, November 1984, 979-984 and in *Liposome Technology*, Volume I, pages 19-27, Gregoriadis, Ed., CRC Press, Inc., Boca Raton, Florida, USA, an immunogen is mixed with a dispersion of an amphiphile in a hydrophilic solvent, such that the amphiphile molecules form an assembly in which the hydrophilic head groups face outwards towards the hydrophilic phase which contains the immunogen. The hydrophilic solvent is then removed to leave a dry composition in which the hydrophilic head groups of the amphiphile molecules are orientated towards the immunogen.

In this first method, it is preferred that the hydrophilic solvent is water although other polar solvents may be used.

The form taken by the amphiphile assembly may be micelles, unilamellar vesicles, preferably small unilamellar vesicles which are generally understood to have a diameter of about 25 nm, multilamellar vesicles or tubular structures, for example cochleate cylinders, hexagonal phase, cubic phase or myelin type structures. The form adopted will depend upon the amphiphile which is used and, for example, amphiphiles such as phosphatidyl choline (PC) tend to form small unilamellar vesicles whereas lyso-phosphatidyl choline forms micelles. However, in all of these structures, the hydrophobic tails of the amphiphile molecules face inwards towards the centre of the structure while the hydrophilic head groups face outwards towards the solvent in which the immunogen is dispersed.

The weight ratio of amphiphile:immunogen will generally be in the region of from 1:1 to 100:1, preferably from 2:1 to 20:1 and most preferably about 8:1 for PC and 4:1 for lyso-PC.

These ratios are preferred ratios only and, in particular, it should be pointed out that the upper limit is set by economic considerations which mean that it is preferable to use the minimum possible amount of amphiphile. The lower limit is somewhat more critical and it is likely that ratios of 2:1 or below would only be used in cases where the immunogen has a significant hydrophobic portion or is exceptionally large.

Good performance is obtained when the solvent is removed quickly and a convenient method for the removal of the solvent is lyophilisation, although other methods can be used.

In some cases, it may be helpful to include salts in the hydrophilic solution, particularly if the immunogen is a macromolecular compound such as a large protein. However, because the presence of larger amounts of inorganic salts tends to give rise to the formation of crystals and, hence, to a cloudy solution, it is preferred that organic salts are used rather than inorganic salts such as sodium chloride. Ammonium acetate is especially suitable for this purpose since it has the additional advantage that it is easily removed by freeze drying.

A second method for the preparation of a composition containing an array of amphiphiles with their head groups pointing towards the moieties of the immunogen is to co-solubilise the immunogen and the amphiphile in a common solvent followed by removal of the solvent.

Therefore, in another preferred embodiment of the second aspect of the invention there is provided a process for preparing a vaccine which comprises co-solubilising an immunogen and an amphiphile in a common solvent and subsequently removing the common solvent thereby forming an immunogen/amphiphile array wherein the hydrophilic head groups of the amphiphile molecules are orientated towards the immunogen.

When this method is used, it is preferred that the weight ratio of amphiphile:immunogen is from about 1:1 to 50:1,

preferably from 2:1 to 10:1 and most preferably about 4:1.

The common solvent must, of course, dissolve both the amphiphile and the immunogen and will, for preference, be a polar organic solvent such as dimethylformamide, dimethylsulphoxide or, most suitably, glacial acetic acid.

In this method, in contrast to the first method, it is unlikely that an array will be formed before the removal of the common solvent.

It seems probable that, on removal of the solvent, the amphiphile molecules tend to order themselves in sheets with their head groups towards the immunogen and their lipophilic tail groups facing away from the immunogen. However, the effectiveness of the present invention does not depend on the accuracy or otherwise of this observation.

It has been observed that good results are obtained when the solvent is removed slowly, for example by drying under a stream of nitrogen, probably because this allows more time for the amphiphile molecules to reorder themselves.

Any hydrophobic solvent for the amphiphile may be used, but for the water-in-oil emulsions preferred for use with small immunogens, a low boiling point solvent such as diethyl ether is preferred since it has been found that the best results are obtained when the hydrophobic solvent is removed slowly by gentle methods such as evaporation and, clearly, this is most effective using a

low boiling point solvent. Low boiling point solvents are also preferred for water-in-oil emulsions although, for these, lyophilisation is a more suitable method of solvent removal. The hydrophilic solvent will preferably be aqueous.

The weight ratio of amphiphile:immunogen may be from about 1:1 to 50:1, preferably from 2:1 to 10:1 and most preferably about 4:1.

The ratio of hydrophilic solution to hydrophobic solution is not critical, but if small immunogens are used, it is preferably such as to ensure the formation of a water-in-oil emulsion rather than an oil-in-water emulsion.

An alternative method of forming the array, which may be particularly suited to use with small immunogens, is to entrap the immunogen in closed lipid vesicles such as small unilamellar vesicles (SUVs) dispersed in a hydrophilic solvent and then to remove the solvent.

One example of an amphiphile which is not capable of forming liposomes is lyso-lecithin. In most aqueous environments, this amphiphile forms micelles rather than small unilamellar vesicles and it is therefore unsuitable for use in the preparation of liposomes. It is however extremely useful in the process of the present invention, particularly when used in conjunction with a compatible hydrophobic solvent such as oleic acid.

In a third aspect, the present invention provides a single phase hydrophobic preparation of an immunogen in a hydrophobic solvent, obtainable using the methods described herein.

In a fourth aspect, the invention provides a two phase composition comprising a hydrophilic phase and a hydrophobic phase wherein the hydrophilic phase comprises an immunogenic composition of the invention. In particular, the hydrophobic phase is dispersed in a continuous hydrophilic phase, and is preferably an emulsion.

In a fifth aspect the present invention provides the use of an immunogen dissolved in a hydrophobic solvent in which it would not normally be soluble in the preparation of an immunogenic composition, particularly an oral vaccine.

In a sixth aspect, the invention provides an enteric coated capsule containing an immunogenic composition or a single phase hydrophobic preparation of the invention.

One advantage of the single phase preparations described above is that they are essentially anhydrous and therefore stable to hydrolysis. They are also stable to freeze-thawing and have greater stability at high temperatures, probably because water must be present in order for the protein to unfold and become denatured. This means that they may be expected to have a much longer shelf life than aqueous preparations of the immunogens.

The invention will now be further described with reference to the following examples, which should not be construed as limiting the invention.

The examples refer to the figures in which:

FIGURE 1: shows response to tetanus toxoid after oral administration of a formulation of the invention; and

FIGURE 2: shows response to tetanus toxoid after subcutaneous administration of a formulation of the invention.

EXAMPLE 1

1 ml of tetanus toxoid, at a concentration of 3000 Lf/ml (6 mg/ml) was dialysed overnight against 1 litre of distilled water. Soya phosphatidyl choline was dispersed in distilled water by probe sonication for 10 minutes with cooling at a concentration of 100 mg/ml. 1 ml of this solution was dispensed into a glass screw-capped 2 ml vial, and 40 µl of tetanus toxoid (5 mg/ml after dilution) was added with mixing. The mixture was lyophilised overnight, and 1 ml of oleic acid added. A crystal clear solution was obtained, which was stored frozen until required for use.

100 µl of this preparation (referred to as formulation 'A') was administered either subcutaneously or through an intragastric tube to inbred young adult Swiss mice (20 µg tetanus toxoid per animal, 3-4 mice per group) housed three to a cage under controlled conditions and fed with food and water *ad lib*. Plasma samples were taken two weeks after administration from the tail vein, and the antibody (IgG) levels against tetanus antigen measured by sandwich ELISA at a 1:100 dilution. The results, expressed as optical density at 450 nm, are shown in

figures 1 and 2.

EXAMPLE 2

Soya phosphatidyl choline was dispersed in distilled water by probe sonication for 10 minutes, with cooling, at a concentration of 100 mg/ml. 1 ml of this solution was dispensed into a glass screw-capped 2 ml vial, and 40 µl of tetanus toxoid, as in Example 'A', was added with mixing. The mixture was lyophilised overnight, and 1 ml of Miglyol 818 was added. A crystal clear solution was obtained, which was stored frozen until required for use.

100 µl of this preparation (referred to as formulation 'B') was administered either subcutaneously or through an intragastric tube to inbred young adult Swiss mice (20 µg tetanus toxoid per animal, 3-4 mice per group) housed three to a cage under controlled conditions and fed with food and water *ad lib*. Plasma samples were taken two weeks after administration from the tail vein, and the antibody (IgG) levels measured by sandwich ELISA at a 1:100 dilution. The results, expressed as optical density at 450 nm, are shown in figures 1 and 2.

EXAMPLE 3

Soya phosphatidyl choline was dispersed in distilled water by probe sonication for 10 minutes with cooling at a concentration of 100 mg/ml. 1 ml of this solution was dispensed into a glass screw-capped 2 ml vial, and 40 µl of tetanus toxoid, as in Example 'A', was added with mixing. The mixture was lyophilised overnight, and 1 ml of a commercial source of sunflower oil was added. A crystal clear solution was obtained, which was stored

frozen until required for use.

100 µl of this preparation (referred to as formulation 'C') was administered either subcutaneously or through an intragastric tube to inbred young adult Swiss mice (20 µg tetanus toxoid per animal, 3-4 mice per group) housed three to a cage under controlled conditions and fed with food and water *ad lib*. Plasma samples were taken two weeks after administration from the tail vein, and the antibody (IgG) levels measured by sandwich ELISA at a 1:100 dilution. The results, expressed as optical density at 450 nm, are shown in figures 1 and 2.

EXAMPLE 4

Hexadecyl phosphoryl choline was dissolved in distilled water at a concentration of 100 mg/ml. 500 µl of this solution was dispensed into a glass screw-capped 2 ml vial, and 20 µl of tetanus toxoid, as in Example 'A', (5 mg/ml after dilution) was added with mixing. The mixture was lyophilised overnight, and 500 µl of oleic acid added. A crystal clear solution was obtained, which was stored frozen until required for use.

100 µl of this preparation (referred to as formulation 'D') was administered through an intragastric tube to inbred young adult Swiss mice (20 µg tetanus toxoid per animal, 3-4 mice per group) housed three to a cage under controlled conditions and fed with food and water *ad lib*. Plasma samples were taken two weeks after administration from the tail vein, and the antibody (IgG) levels measured by sandwich ELISA at a 1:100 dilution. The results, expressed as optical density at 450 nm, are shown in figure 1.

EXAMPLE 5

β-octyl glucoside was dissolved in distilled water at a concentration of 100 mg/ml. 500 µl of this solution was dispensed into a glass screw-capped 2 ml vial, and 20 µl of tetanus toxoid, as in Example 'A', (5 mg/ml after dilution) was added with mixing. The mixture was lyophilised overnight, and 500 µl of glycerol monooleate was added. A crystal clear solution was obtained, which was stored frozen until required for use.

100 µl of this preparation (referred to as formulation 'E') was administered subcutaneously to inbred young adult Swiss mice (20 µg tetanus toxoid per animal, 3-4 mice per group) housed three to a cage under controlled conditions and fed with food and water *ad lib*. Plasma samples were taken two weeks after administration from the tail vein, and the antibody (IgG) levels measured by sandwich ELISA at a 1:100 dilution. The results, expressed as optical density at 450 nm, are shown in figure 2.

EXAMPLE 6

The amphiphile β-octyl glucoside was dissolved in distilled water at a concentration of 100 mg/ml. 500 µl of this solution was dispensed into a glass screw-capped 2 ml vial, and 20 µl of tetanus toxoid, as in Example 'A', (5 mg/ml after dilution) was added with mixing. The mixture was lyophilised overnight, and 500 µl of phytol was added. A crystal clear solution was obtained, which was stored frozen until required for use.

100 µl of this preparation (referred to as formulation

'F') was administered subcutaneously to inbred young adult Swiss mice (20 μg tetanus toxoid per animal, 3-4 mice per group) housed three to a cage under controlled conditions and fed with food and water *ad lib*. Plasma samples were taken two weeks after administration from the tail vein, and the antibody (IgG) levels measured by sandwich ELISA at a 1:100 dilution. The results, expressed as optical density at 450 nm, are shown in figure 2.

CLAIMS

1. An immunogenic composition comprising an immunogen solubilised, or otherwise distributed, in a hydrophobic solvent in the absence of a hydrophilic phase.

2. An immunogenic composition as claimed in claim 1 wherein the immunogen is solubilised in the hydrophobic solvent to form a single phase.

3. An immunogenic composition as claimed in claim 1 or claim 2, wherein the immunogen is an antigen.

4. An immunogenic composition as claimed in any one of claims 1 to 3 which is a vaccine or can be used in the preparation of a vaccine.

5. An immunogenic composition as claimed in claim 4 wherein the vaccine is an oral vaccine.

6. An immunogenic composition as claimed in any one of claims 1 to 5 wherein the immunogen is selected from Diphtheria toxoid, tetanus toxoid, botulin toxoid, snake venom antigens, Hepatitis B antigens, whooping cough subunit, influenza a and/or b (or whole killed virus or protein subunits), cholera antigens, *H. pylori* antigens, whole bacteria, or extracts thereof, e.g. *P. aeruginosa*, *chlamydia* species, *neisseria* species, *yersinia* species, *salmonella* species, fungi or fungal antigens, e.g. from Candida, rabies virus, polio virus, rotavirus, measles virus, rubella, respiratory syntitial virus, HIV, BCG, other mycobacterial antigens, *H. influenza* A or B (with or without carrier protein), protozoal antigens, e.g. malaria, leishmania, toxoplasma, trypanosoma, trematode

antigens, e.g. schistosoma, cestode antigens, e.g. from cysticerca, echinococcus, nematode antigens, e.g. toxocara, hookworm and filaria, spirochete antigens, e.g. *borrelia* species, surface membrane epitopes specific for cancer cells, and cell receptor targeting anti-inflammatory immunomodulators (for treatment of inflammatory diseases such as Crohn's Disease and rheumatoid arthritis), e.g anti TNF-R and anti IL-1R, polysaccharides or polymer conjugates of steroids.

7. An immunogenic composition as claimed in any one of claims 1 to 6 wherein two or more antigens are co-presented in the composition.

8. An immunogenic composition as claimed in claim 7 wherein a protein antigen and a polysaccharide antigen are co-presented.

9. An immunogenic composition as claimed in any one of claims 1 to 8, wherein the immunogen is a peptide, polysaccharide or other antigen conjugated to at least one medium- or long-chain hydrocarbon tail.

10. An immunogenic composition as claimed in any one of claims 1 to 9 wherein one or more additional immuno-stimulants is present.

11. An immunogenic composition as claimed in claim 10 wherein the additional immuno-stimulant(s) is/are selected from IL-4, IL-10, IL-12, γ-interferons, monophosphoryl lipid A, mycobacterial extracts, muramyl dipeptide and analogues thereof, tuftsin and cholera subunit B, and heat labile toxin of *E. coli*.

12. An immunogenic composition as claimed in any one of claims 1 to 3 wherein the composition will cause a reduction in the elimination of an immune response against the antigen.

13. An immunogenic composition as claimed in claim 12 wherein the antigen is an HLA antigen or DNA.

14. An immunogenic composition as claimed in any one of claims 1 to 3, wherein the composition will desensitise the immune response.

15. An immunogenic composition as claimed in claim 14 wherein the antigen is selected from pollens, dust mite antigens or food allergies.

16. A process for the preparation of an immunogenic composition which comprises the step of solubilising, or otherwise distributing, an immunogen in a hydrophobic solvent in which it would not normally be soluble.

17. A process as claimed in claim 16 which comprises the following steps:

(i) associating the immunogen with an amphiphile in a liquid medium;

(ii) removing the liquid medium to leave an array of amphiphile molecules with their hydrophilic head groups orientated towards the immunogen and wherein there is no chemical interaction between the amphiphile and the immunogen; and

(iii) providing a hydrophobic solvent around the

immunogen/amphiphile array.

18. A process as claimed in claim 16 or claim 17 modified by any one or more of the features of claims 3 to 15.

19. A process as claimed in claim 17 or claim 18 wherein the amphiphile is a phospholipid, an octyl glucoside, α-tocopherol, or esters thereof, or a bile salt, or mixtures thereof.

20. A process as claimed in claim 19 wherein the phospholipid has a phosphatidyl choline head group.

21. A process as claimed in claim 20 wherein the phospholipid is phosphatidyl choline (PC), lyso-phosphatidyl choline (lyso-PC), sphingomelcin, a derivative of one of the above such as hexadecyl phosphocholine or an amphiphile polymer containing phosphoryl choline.

22. A process as claimed in any one of claims 16 to 21 wherein the hydrophobic solvent comprises a hydrocarbon, e.g. mineral oil or squalene, a long chain fatty acid, a medium chain alcohol, a polyol, e.g. α-tocopherol, a medium- or long-chain mono-, di- or tri-glyceride, or mixtures thereof.

23. A process as claimed in any one of claims 17 to 22 wherein the amphiphile comprises PC and the hydrophobic solvent is a triglyceride, or wherein the amphiphile comprises lyso-PC and the hydrophobic solvent is oleic acid.

24. A process as claimed in any one of claims 17 to 22, wherein the immunogen/amphiphile array is formed by mixing the immunogen with a dispersion of an amphiphile in a hydrophilic solvent and removing the hydrophilic solvent.

25. A process as claimed in claim 24, wherein the hydrophilic solvent is water.

26. A process as claimed in claim 24 or claim 25, wherein the amphiphile assembly comprises micelles, unilamellar vesicles, for example unilamellar vesicles, multilamellar vesicles or a tubular structure such as cochleate cylinders, hexagonal phase, cubic phase or myelin type structures.

27. A process as claimed in any one of claims 24 to 26, wherein the hydrophilic solvent is removed by lyophilisation.

28. A process as claimed in any one of claims 17 to 24, wherein the immunogen/amphiphile array is formed by co-solubilising the immunogen and the amphiphile in a common solvent and subsequently removing the common solvent.

29. A process as claimed in claim 28, wherein the weight ratio of amphiphile to immunogen is from about 1:1 to 50:1.

30. A process as claimed in claim 29, wherein the hydrophobic solvent is a low boiling point organic solvent such as diethyl ether.

31. A single phase hydrophobic preparation of an

immunogen in a hydrophobic solvent, obtainable by a process as claimed in any one of claims 17 to 30.

32. A two phase composition comprising a hydrophilic phase and a hydrophobic phase wherein the hydrophobic phase comprises a composition as claimed in any one of claims 1 to 15 or a preparation as claimed in claim 31.

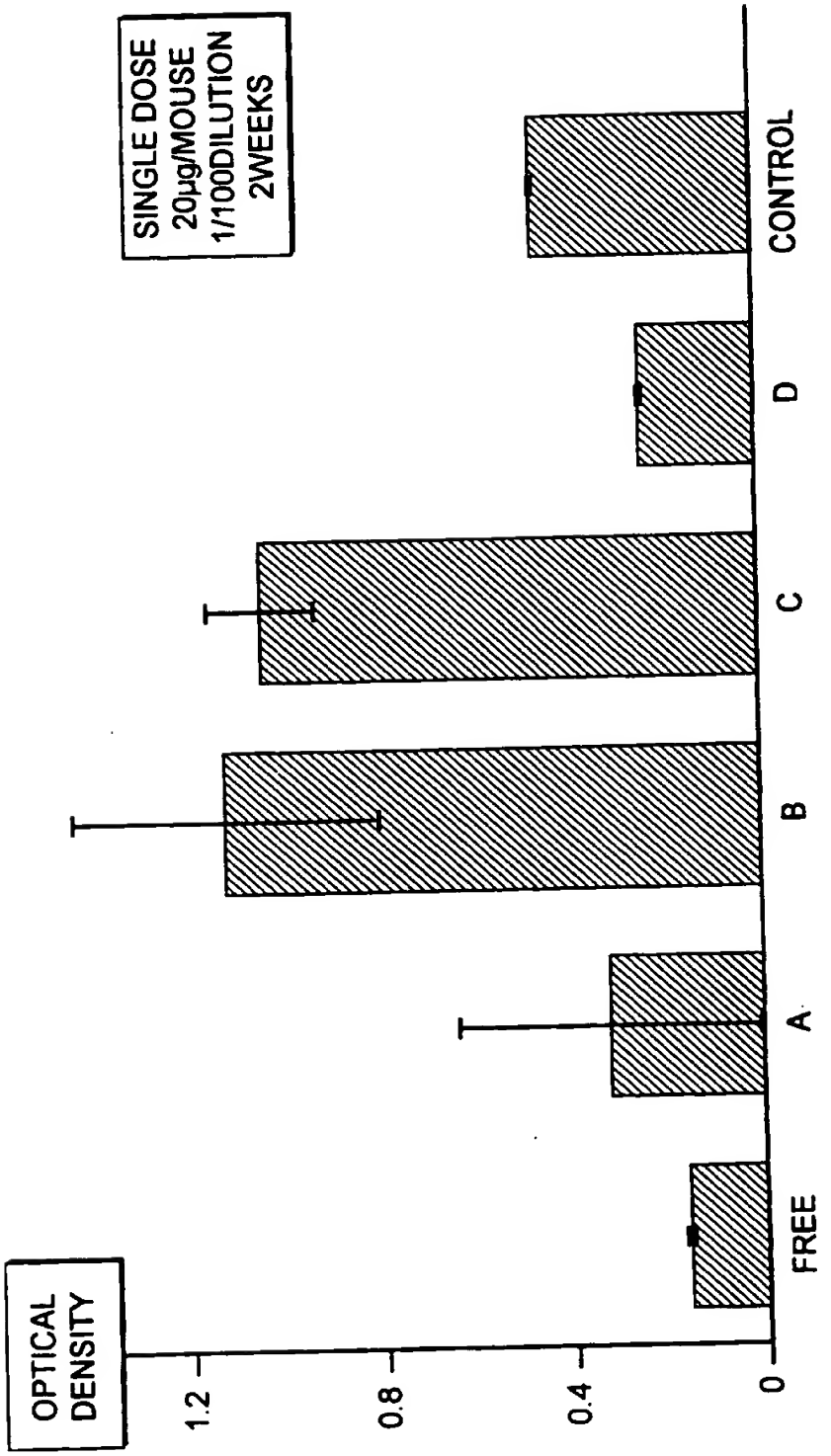
33. A composition as claimed in claim 32, wherein the hydrophobic phase is dispersed in a continuous hydrophilic phase.

34. A composition as claimed in claim 32 or claim 33, which is an emulsion.

35. The use of an imunogen dissolved in a hydrophobic solvent in which it would not normally be soluble, in the preparation of an immunogenic composition.

36. The use as claimed in claim 35, wherein the immunogenic composition is an oral vaccine.

37. An enteric coated capsule containing an immunogenic composition as defined in any one of claims 1 to 15 or a single phase hydrophobic preparation as defined in claim 31.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1.2
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0.4
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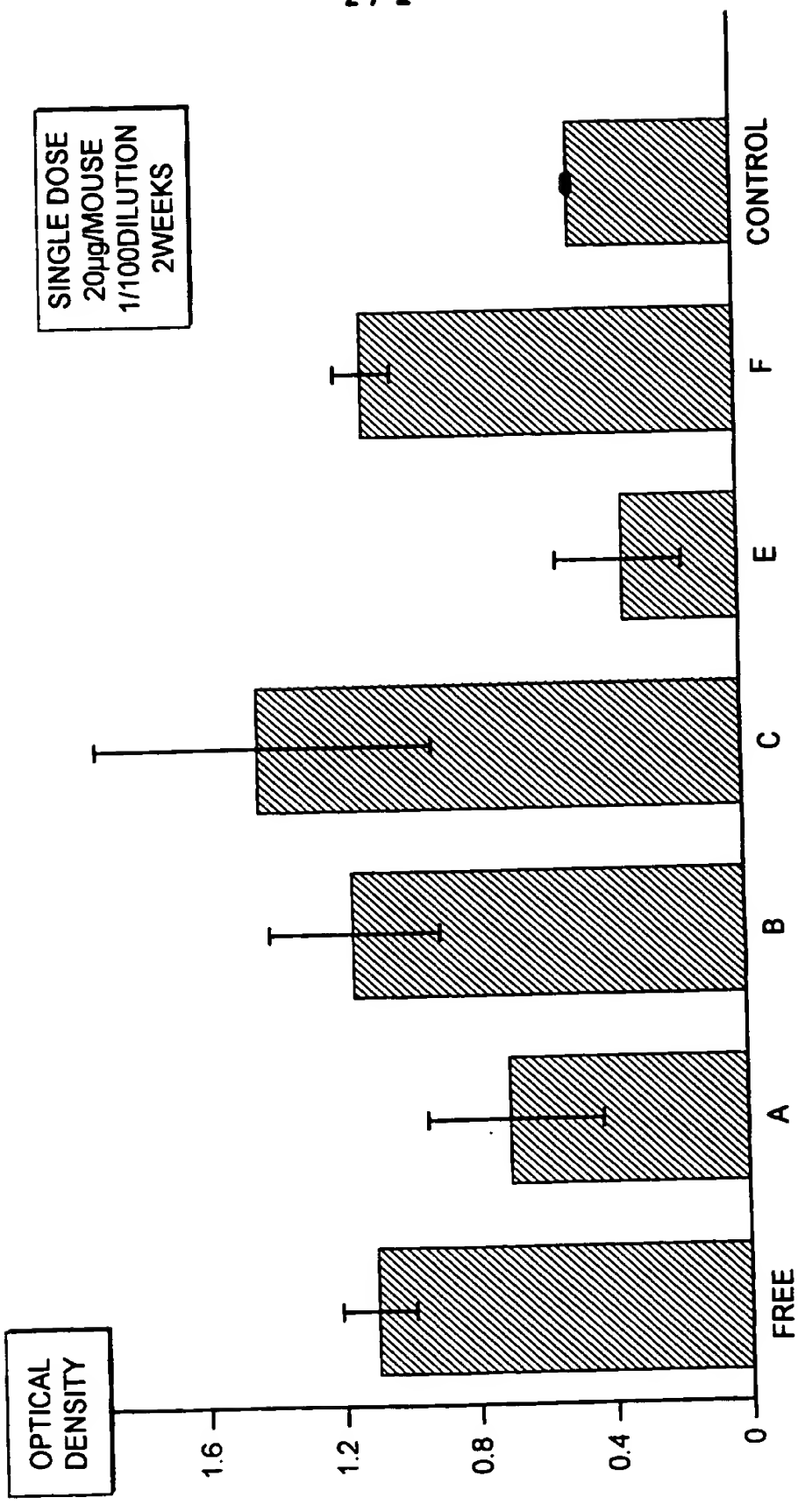
FREE
A
B
C
D
CONTROL

FIG.1


SINGLE DOSE
20µg/MOUSE
1/100DILUTION
2WEEKS
OPTICAL DENSITY
0
0.4
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1.2
1.6
FREE
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F
CONTROL

FIG.2

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 95/02675

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/44 A61K39/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,95 13795 (CORTECS LIMITED) 26 May 1995 cited in the application see the whole document	1-37
A	EP,A,0 521 562 (BROCADES PHARMA B.V.) 7 January 1993 see the whole document	1-37
A	US,A,5 340 588 (DOMB, A.J.) 23 August 1994 cited in the application see the whole document	1-37
A,P	WO,A,95 04524 (OPPERBAS HOLDING B.V.) 16 February 1995 see page 16 - page 28; claims 1-24	1-37

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 February 1996	15.03.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Olsen, L

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/GB 95/02675

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